# FISHERIES PROCESSING BIOTECHNOLOGY– 3 (2 – 1)

## MARINE VERTEBRATA ,INVERTEBRATA AND FISH WASTES AS SOURCES BIOACTIVE AND BIOFUNCTIONAL COMPOUNDS

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- Indonesia is an archipelagic tropical country
- comprising 17508 islands
- The highest biodiversity especially coastal and marine resources
- Indigenous knowledge on traditional medicine

# WHY MARINE ORGANISM

- Most marine organisms produce several secondary metabolites, which, although, are not directly involved in central physiological functions, yet contribute to their fitness and survival.
- many cell compounds of marine organisms also possess certain characteristic bioactivities.
- Several marine organisms have adapted themselves through symbiotic association among themselves that help them survive under harsh environments

# SECONDARY METABOLITES

- Secondary metabolites (also called natural products) are organic compounds that are not directly involved in the normal growth, development, or reproduction of organisms.
- The function of these compounds is usually of an ecological nature, as they are used as defenses against predators, parasites, and diseases for interspecies competition and to facilitate reproductive processes (attracting by color, smell, etc.)

# WHAT ARE BIOACTIVE COMPOUNDS & BIOFUNCTIONAL COMPOUNDS?

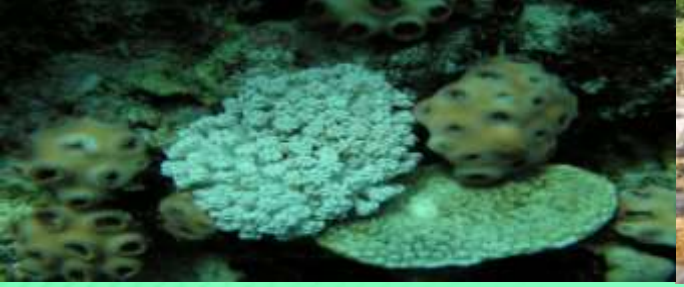
# BIOACTIVE COMPOUNDS

- Chemical compounds produced by living organism that exert a biological effect on other organism include therapheutic activity, for dissease of human & animal, Toxic activities, biodegradable activity.
- Activities:
  - + Anticancer
  - + Antitumor
  - + Anti-HIV
  - + Antibiotic, antifungi, etc

# BIOFUNCTIONAL COMPOUNDS

- Natural compounds that have function on nutritional effects and health benefit.
- Activities:
  - + Antioxidant
  - + Antiobesity
  - + Antidiabetic
  - + Antiaging
  - + Antiinflamatory
  - + Etc.

# SOURCES OF BIOACTIVE COMPOUNDS



- **Marine sponge**
- Jelly fish
- Crustacean
- Tunicates
- **Echinoderms**
- Molluscs
- Bryozoans
- Macro & micro algae

- Marine bacteria & Fungi
- **Marine Toxin**
- Marine Nucleosides
- Marine alkaloids
- Marine Peptides
- Marine Prostaglandin
- **Marine waste**

# MARINE TOXIN

- Paralytic Shellfish Poisoning
  - Saxitoxin (14 jenis)
- Tetrodotoxin
- Neurotic Shellfish Poisoning
  - Brevetoxin (2 jenis)
- Ciguatera (seafood Poisoning)
  - Ciguatoxin - palytoxin
  - Brevetoxin - gambierol
  - Maitoxin - gambieric Acids

# MARINE TOXIN

- Diarrheic Shellfish Poisoning
  - Okadoic Acids - Yesotoxin
  - Pectonotoxin

- Miscellaneus toxin
  - Amphidinolides - Gonoidomin A
  - Amphidinol - Surugatoxin
  - Prorocentrolide - Neosurigatoxin
  - Aplysiatoxin & Debromoaplysiatoxin
  - Toxic Peptides

# MARINE WASTE

- Chitin, chitosan & chitosan oligosaccharides
- Glucosamine
- Gelatin
- Fish bone

# BIOACTIVE COMPOUNDS

# BIOACTIVE METABOLITES FROM MARINE INVERTEBRATES

- Steroids - Nitrogen heterocyclic
- Terpenoids
- Isoprenoids
- Nonisoprenoids
- Quinones
- Brominated compounds
- Nitrogen sulphur heterocyclic

# BIOACTIVE METABOLITES FROM MARINE ALGAE

- Brominated Phenols
- Oxygen Heterocyclics
- Sterol
- Terpenoids
- Polysaccharides
- Peptides
- Nitrogen sulphur heterocyclic
- Proteins

# BIOACTIVE METABOLITES FRO MARINE ALGAE

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- Proteins

# BIOACTIVE COMPOUNDS ACTIVITIES

# 2ND MEETING

# BIOACTIVE COMPOUNDS FROM ECHINODERM

- Saponins → mainly responsible 4 biological activity on echinoderms
- Asterosaponins → are reported have hemolytic, antineoplastic, cytotoxic, antitumor, antibacterial, antiviral, antifungal, & anti-inflammatory activities.
- Asterosaponins (Asa) → sterol derivates
- SC saponins are terpenoid in nature
- Both groups have sulphate ester & quinose sugar moietis.
- The saponins from other sources lack sulphate functionality
- Asa the sulphate function is attached to an aglycone

# SEA CUCUMBER

- Sea cucumbers → found in shallow sea water areas – deep ocean floors.
- Several species of sea cucumbers → possess antibacterial, antifungal, antioxidant, & anticoagulant activities.
- Antibacterial activity → extracted from different body parts and eggs of *Cucumaria frondosa.*
- *Cucumaria frondosa* → *exhibits high antioxidant activity, which is* present in the digestive tract, gonads, muscles, and respiratory organ of the organism.

- body wall of the sea cucumber contains high amounts of sulfated glycans → anticoagulant activity, venous antithrombic activity, & recombinant HIV reverse transcriptase activity.
- these compounds are involved in maintaining the integrity of the sea cucumber's body wall

# BIOACTIVE COMPOUNDS FROM FISH WASTES

# FISH SKIN - COLLAGEN

- Collagen is the major structural protein found in the skin & bones all animals.
- Collagen is commonly used in medical and pharmaceutical
- As cosmetic → bound water → long lasting moisturizing effects.
- Enzymatic collagen from filefish → better emulsifying ability and foamability and an ideal material as a low-fat proteinaceous surfacatant

# GELATIN

- Gelatin is widely used in the pharmaceutical and food industries for encapsulation of drugs and as a food additives to enhance texture, WHC & stability products.
- Gelatin → ↑ glycine, proline & alanine.
- Gelatin → help to improve the structure & health of hair & nails.
- Bioactivities of proteins are attributable to the presence of biologically active peptide sequences in their primary structure

# ANTI-MICROBIAL ACTIVITY

- Various antimicrobial peptides have been used to reduce pathogens in foods & to extend the shelf life of many perishable foods.
- The antimicrobial peptides → prevent growth of *Clostridium botulinum spores in foods such* as cheese & inhibit the growth of *Listeria monocytogenes*.

# GELATIN – ACE-INHIBITORY ACTIVITY

- ACE (Angiotensin I converting enzyme (ACE) → plays an instrumental role in elevation of blood pressure.
- 2 peptides were purified from Alaska pollack skin gelatin extract having ↑ ACE-inhibitory activity
- ACE inhibitors lower blood pressure by inhibiting ACE, key comp. of renin angiostensin system, whose main function is to convert Ang I to Ang II.
- that activitiy has been demonstrated to be beneficial in modifying human disease progression including hypertention.

# GELATIN – ANTIOXIDANT ACTIVITY

- Ox causes many unfavorable impact on food & biological system.
- Oxidation is associated with the occurrence of sev disease conditions including atherosclerosis, inflammation, & cancer.
- Antiox ➔ overcome oxidation-mediated problems.
- Alaska pollack  gelatin have potential as safe & potent natural antioxidant.

# CHITIN AND CHITOSAN

- Chitin & chitosan have a variety of nutraceutical applications, including immunenhancement, dissease recovery, & dietary fiber.
- Their abilities depend on molecular weight & degree of N-acetylation -→ greatly influence solubility along with interaction to other biomolecules.

# ANTIMICROBIAL ACTIVITY

- Chi & Cho have been reported against a range of fungi, yeast & bacteria.
- Cho has greater antimicrobial activity than Chi → it is possitive charge of ↓ pH, allowing it to disrupt the negatively charge cell membranes of bacteria.
- Cho is known 4 its ability to chelate trace material → help to inhibit microbial growth & production of toxin

- Cho against microorganisms including *Aeromonas hydrophila, Bacillus cereus, B. licheniformis, B. subtilis, Clostridium perfringens, Brochothrix spp., Lactobacillus spp., Listeria monocytogenes, Pseudomonas spp., Salmonella typhimurium, S. enteritidis, Serratia liquifaciens,* and others
- Yeast (*Candida spp. and Saccharomyces spp.)*
- *mold (Aspergillus spp., Penicillium spp., and Rhizopus spp.) in a variety of foods*

# MECHANISM

- Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. The mechanism of action appears to derive in part from the ionic interaction between the cationic groups of the chitosan molecules and the anionic groups of the microbial cell membrane, which can rupture the cell membrane.

# ANTI-INFLAMMATORY ACTIVITY

- Inflammation is the basic immune response of the body to injury, infection, or irritation, & it is characterized by symptoms such as redness, heat, swelling, pain & organ dysfunction.
- Cho is able to inhibit proinflamatory agents.
- Cho was able to prevent pulmonary inflammation by inhibiting type 2 helper T cells (Th2) & reducing levels of interleukin – 4 (IL-4) & IL-5 ➔ impt compt of the immune response to allergens.
- Water soluble of Cho ➔ inhibiting tumor necrosis factor-α (TNF-α) & IL-6, 2 proinflamation cytokines, in human astrocytoma cells.
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- TNF-α → allergic inflamation
- Cho is partially inhibit TNF-α & IL-6 from mast cell.
- WS Cho is reduce allergic inflammatory response by down-modulating the $Ca2^{+}$ - induced act of mast cells.
- Mast cell → necessary 4 allergic reactions & have been implicated in a number of neuroinfalammatory diseases.

# ANTIOXIDANT ACT

- Reactive oxygen species (ROS) and free radicals → naturally generated in the body during aerobic metabolism and can cause oxidation of lipids, proteins, sugars, sterols, and nucleic acids.
- ROS activity → associated with a number of age-related health disorders, including arthritis, cancer, stroke, atherosclerosis, retinal damage, and heart attack.
- Antioxidative nutraceuticals, such as tocopherols, ascorbic acid, carotenoids, polyphenols, and possibly chitosan → minimize oxidative damage & reduce the risk for age-related disorders by preventing the accumulation of ROS and free radicals

- Kinds of antioxidants (Agullo et al,).
- Primary antioxidants, which involve the use of a phenol group during the initial part of the oxidative reactions.
- Secondary antioxidants, which can chelate metal ions that catalyze the oxidative reactions.
- ↓ MW chitosan > effective at scavenging superoxide and hydroxyl radicals compared to ↑ MW chitosan, with sulfated chitosans showing the greatest effect

# ANTI-CARCINOGENIC ACTIVITY

- nutraceuticals → ↓ oxidative stress & subsequent DNA damage, some nutraceuticals can adsorb mutagens and thereby inhibit their carcinogenic activity.
- Most of the anticarcinogenic studies → focused on their oligomers, which have been shown to inhibit heavy metal–induced genotoxicity and possess growth inhibitory and antimetastatic effects against a variety of cancerous tumors.
- Chi & Cho were successfully adsorbed in both ionic & nonionic solutions

# APPLICATION IN BIOTECHNOLOGY

- Cho is used as a matrix for immobilization of enzymes and also as whole microbial cells.
- metabolite control in artificial organs.
- Potential for production of hydrogen by fermentation of marine.

# 3RD MEETING

# SEPARATION AND ISOLATION TECHNIQUES

# STEPS OF FINDING NEW DRUGS

- Separation
- Fractionation
- Isolation
- Screening
- Toxicity evaluation
- Clinical trial
- Publish

# SEPARATION TECHNIQUES

# WATER SOLUBLE CONSTITUENTS

- There are number of problems associated with separation of water soluble compounds (WSC).
- The abundance of salt , make separation more perplexing.
- Bacterial& fungal might growth during aqueus fraction of ethanolic/methanolic extract → often degrades antitumor activities.
- Desalting → most important & difficult process.
- Generally most desalting methods are not applicable 4 isolation of ↓ MW compounds of aqueous extract of marine org.
- Desalting using methanol → conveniently & effectively
- There is no established standard fractionation procedure 4 WSC & often rely on trial & error.

# ION EXCHANGE CHROMATOGRAPHY

- Most effective methods 4 separating WSC → ionic character of compounds & stability on resin & buffer are known.
- Resin depends on ionic character & stability of target compounds.
- $H^+$ → strongly acidic resin; $OH^-$ → strongly basic resin.
- Strong pH 4 elution → often causes decomposition.
- Use weakly acidic / basic resin / poorly buffered resins → preferred.
- Resins with medium acidity /basicity are available.

# REVERSE-PHASE (RP) COLUMN

- RP used 4 separation of compounds with a wide range of polarity.
- It use RP with various hydrophobic stationary phase with combination: methyl alcohol, acetonitrile, & buffers.
- Problems:
  - 1. sample size is very limited → done 4 final purification / fine separation.
  - 2. use of buffer solution → use buffer with appropriate pH & ionic strengths is often unavoidable -- > overcome: use volatile buffers which can remove by vacuum evaporation

# COMBINATION OF ION EXCHANGE AND SIZE-EXCLUSION CHROMATOGRAPHY

- Attachment of ion-exchange capabilities of matrices of various pore sizes provides a very powerful separation capability.
- Actual separation is due to the combination three principles: ion-exchange, size-exclusion, hydrophilic/hydrophobic interaction.
- The selection of matrix may be the key for the successful separation.
- Strongly acidic or basic resins are also widely used to separate on neutral and amphoteric compounds.
- The key of fractionation method is the use of bioassay to monitor the activity of all fraction produced, so subsequent work is done only on the active fractions.

| Water soluble compounds | Support |
|---|---|
| Mono- and oligosaccharides | Sephadex G-10, G-15, Bio-Gel P-2, strong cation exchange(SO3OH) resins, weakly basic anio exchange resin, e.g. –(CH2)NH2 |
| Polysaccharides | Sephadex G-50-200, Bio-Gel P-2, hydroxyl appatite, DEAE bounded gels. |
| Oligopeptides | Sephadex G-10, -15, Bio-Gel P-2, P-10, Sephadex LH-20, RP HPLC ($C_8$, $C_{18}$). |
| Amino, amino acids, guanidine | Strong cation exchange resins (-SO3-) weak cation exchange resins (-CO2H) RP HPLC ($C_8$ - $C_{18}$ CN)etc |
| Nucleic acids | Anion exchange resins, RP HPLC ($C_8$, $C_{18}$) |
| Polar carboxylic acids | Strong or weak anion exchange resins RP HPLC ($C_{18}$) |
| Glycosides | Sephadex G-10, LH-20, RP ($C_8$, $C_{18}$), XAD-2, XAD-7 |

# FRACTIONATION

- Fractionate using solvent partition is the active extract at early stages.
- Broad fraction are further fractionated by column chromatography (absorption on silica gel, ion exchange, partition, gel permeation) → variety of solvent systems adapted to the polarity of the active fraction.
- Multiple chromatographies → necessary b4 active fraction can be concentrated.
- TLC, HPLC, counter-current distribution, elektrophoresis, & fractional crystalixation → required 4 final phase of isolation.
- The presence of multiple active compounds → difficult to separate → complicates isolation procedures.

# CHARACTERIZATION OF ACTIVE COMPOUNDS

- Knowledge of biosythesis of secondary metabolites is very helpful to deducing the most logical pattern.
- $^{1}H$ NMR, $^{13}C$ NMR, IR, UV, & mass spectra → determined & compared → related basis of chemical & biosynthesis reasons.
- classical methods struc. det. → degradation of molecules to establish of molecules & various transformation reactions combined with rigorous analysis of spectral data of the derivates.
- X rays christalographys → undertake compund & Heavy atom to establish the structure & stereoschemistry

# ISOLATION PROCEDURES

# AMINO ACIDS & SIMPLE PEPTIDES

- These compounds have been isolated from marine algae/terrestrial plants.
- extract material is homogenized with 70 % aqueosu ethanol.
- The extract contain N containing compounds.
- The aqueous phase is partitioned with ether
- The most versatile & efficient method 4 isolation of AA is IEC.
- Aqueous phase may be directly examined 4 AA, but it is advisable to carry out preliminary separation of AA by absorption on strongly acidic ion-exchange resin & subsequent elution with $NH_3$

# PEPTIDES

- Peptides found in algae & sponge
- Discoderma-A, first bioactive peptides from sponge, contains the rare *tert*-leucine cysteic acid & sev D-amino acids.
- HPLC, 2D NMR & FAB spectrometry are used in peptides isolation → presence of blocked N-termini & β & γ-amino acid residues.
- Small amount of peptides are able to detect wit chiral chromatography.
- Peptides isolated from marine sponge were usually cyclic & lipophilic

# NUCLEOSIDES

- The pyrrolo (2,3-d) pyrimidine nucleosides mycalesine a & B were obtained by extraction of the *Mycale* spp with ethanol → fractionation with ethyl acetate & subsequent SiO2 flash chromatography. The active fraction were subjected to low pressure CC on Kiesel gel.
- Methylthioadenosine was isolated from nudribanch Doris varrucosa by extraction with acetone → fractionation acetone extract with ether & n-buthanol → chromatography of the active fraction on Sephadex LH-20 column

# CYTOKININS

- Cytokinins are available in seaweed
- Isolated by IEC & purified by preparative TLC on silica gel HF-254.
- The spot are visualized by exposure to I2 vapor/UV light.
- Solvent 4 TLC : n-BuOH-HOAc-H2O (12:3:5).

# ALKALOIDS

- Marine pyridoacridine alkaloids were significant biological activities such as: antiHIV activity, Ca2+ releasing activity, metal chelating properties & intercalation of DNA.
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# MARINE TOXIN - SAXITOXIN

- Alaskan butter clam is best source of Saxitoxin
- The isolation is fairly simple, but not applicable to other sellfish toxins.:
  - Mixture of toxin is broadly resolved by selective adsorption on Bio Gel P-2 or Sephadex G-15.
  - Toxin fraction is eluted with a dilute acetic acid solution
  - The mixture of toxin is then applied on column of weakly carbolix resin.
  - Acetic acid gradient elution furnish pure toxins in the reserve order of the net positive charge of the molecule.

# BREVETOXINS

- Brevetoxins are caused by Gymnodinium breve.
- Brevetoxins A is of interest → most potent toxin & uniquely binds to sodium channels of the excited membrane.
- Stages of brevetoxin A isolation:
  - Isolation from alga
  - The medium containing the cell were acidified to pH 5,5 & extracted with diethyl ether to give 90 mg of crude brevetoxin
  - Repeated flash chromatography of the crude toxin mixture with 5% methanol in diisoprophyl ether (v/v) .
  - Purity of the various toxins was checked by HPLC.
  - Brevetoxin-B crystalized from acetonitrile as colorless needles.

# TETRODOTOXIN

- Tetrodotoxin → unique chemical structure & specific action of blocking sodium channel.
- Soluble in acidic media & weakly basic medium.
- Stages of tetrodotoxin isolation:
  - Treatment toxin with 0,2N HCl did yield of crystalline→ 1-2 g get from 100 kg puffer ovaries → Hirata's procedure.
  - this toxin can be detected by a highly sensitive tetrodotoxin analyzer which separates them on a reverse-phase column & detects fluorescents products formed upon heating the toxin with sodium hydroxide solution.

# CIGUATOXIN AND ITS CONGENERS

- Ciguatera, endangers public health & hampers local fisheries, is caused by ciguatoxin & its congeners.
- Ciguatoxin was obtained as a white solid.
- Ciguatoxin → polyether compounds
- Ciguatoxin analogs have been found in fish & dinoflagellates.

# BIOLOGICAL ACTIVITY SCREENING

# TYPES OF SCREENING

- Individual activity screening
- Broad biological screening
- Screening model & activity
  - Antifungal
  - Antibacterial
  - Antimalarial
  - Antiviral
  - Antiinflamatory
  - Antihypertensif
  - antiallergic

# ANTIBACTERIAL & ANTIFUNGAL ACTIVITIES

- In vitro antimicrobial testing can be carried out by several methods:
  - Poison food technique
  - Disc diffusion method
  - Tube dilution methods
  - Microtitre technique

# ANTIMALARIAL ACTIVITY

- The secondary screening is conducted in rhesus monkey infected with *P . cynomolgi* Chloroquine ( 5 mg x kg x 7 days) is used as standard drug.
- Absence of any recrudescence upto the end of 50 days indicate complete cure.
- Radical curative activity of the test substance is evaluated in rhesus monkey infected with *P. cynomolgi*

# ANTIVIRAL ACTIVITY

- Antiviral activity must be evaluated in a living cell / animal host.
- Testing for antiviral act. Is usually conducted in cell culture, chicken egg, & animal models.
- In vitro cell culture procedures:
  - The cells are infected with the virus & then exposed to the test substances.
  - If the substance has antiviral activity the multiplication cell will be inhibited → the morphology of the cell monolayer.

# ANTIVIRAL ACTIVITY

- Chicken egg → simple, effective & economical.
- 3 mains routes the test substances could be administered:
  - Allontoic cavity inoculation
  - Amniotic cavity inoculation
  - Choric allantoic membrane (CAM) inoculation
- The virus & test subtance may be given through the same / different route in b4, along with / after virus infection.

# ANTIVIRAL ACTIVITY

- Animal model has relatively maximum predictive among the various methods 4 detecting antiviral act.
- This model can identify both antiviral act & antiviral agent.
- The ideal animal model should have 3 features:
  - Use of human virus
  - Use of natural route of infection & size of inoculum as in man
  - Similarity of infection, pathogenesis, hsot response, drug metabolism & drug toxicity

# ANTIINFLAMATORY ACTIVITY – ACUTE MODELS

- According to Srimal et al 1971 (carrageenin-induced Oedema in mice:
  - Carr solution saline (0,025 ml of 1.0 %) is injected subcutanneously into the hind paw of mice with the help of Hamilton microsyringe after 1 h of oral feeding material.
  - The mice are killed after 3 h with an overdose of ether.
  - Both the hind paws are cut identically at the ankle of joint & weighed.
  - The difference between the weight of the 2 paws gives the amount of oedema developed in that particular animal.
  - The mean of group is calculated & compared with the mean of oedema developed in control group.
  - Test material showing less than 20% activity are rejected.

# ANTIINFLAMATORY ACTIVITY – ACUTE MODELS

- Carrageenin-induced Oedema in Rats:
  - Carr (0.1 ml of 1 % solution) is injected in the hind paws.
  - Vol of the paw is measured plethysmographically immediately & 3 h after injection of the irritant.
  - The difference in the volumes give the amount of oedema developed.
  - Percent inhibition of the oedema between the control group & the test substance treated group is calculated & compared with the group receiving standard drug.

# ANTIINFLAMATORY ACTIVITY – SUB-ACUTE MODELS

- Cotton Pellet Test
  - Test autoclaved pellet of cotton / sponge (50° + 1 g) are implanted on the saved back of rats aseptically, 1 on each side of the midline incision.
  - The test material is fed once a day from day one to seven of the experiment.
  - On the 8 d the rats are sacrificed by a overdose of ether.
  - The pellets surrounded by granuloma tissue are dissected out carefully & dried in hot oven at 60°C till a constant weight is obtained.
  - Percent inhibition compared to the control group is calculated.

# ANTI-INFLAMMATORY ACTIVITY – SUB-ACUTE MODELS

- Granuloma pouch test
  - Pouch on the back of rats is produced by injecting air (20 ml) in the subcutaneous tissue followed by 1.0 ml of 1.0% croton oil in sterile olive oil.
  - The test substance is fed from 1$^{st}$ – 13$^{th}$ day of experiment, and rats are sacrificed on the 14$^{th}$ day.
  - The exudate formed in the pouch is aspirated & measured.
  - The pouch itself is dissected out carefully & dried at 60°C to constant weight.
  - The percent of inhibition compared to the control group is calculated.

# ANTI-INFLAMMATORY ACTIVITY – SUB-ACUTE MODELS

- Formaldehyde-induced Arthritis
  - Formaldehyde (0.1 ml / 2.0% formaldehyde solution) is injected into the hind paws of rats on 1st d, & 3rd d of exp.
  - Vol of the paw is measured b4 the injection of the irritant & once daily 4 next 10 d.
  - The test substances is fed once a day from 1st – 10th of the exp.
  - The mean increase in the paw vol of each group over period of 10 d is calculated & compared with the control group to find the difference

# ANTI-INFLAMMATORY ACTIVITY – CHRONIC MODELS

- Adjuvant-induced Arthritis
  - Killed *Mycobacterium tuberculosis* (0.5 mg) suspended in liquid paraffin (0.1 ml) are injected into one of the hind paws of the rat.
  - Vol of both the paws is measured plethysmographically daily 4 next 10 d.
  - The test substance can be administered either from 1$^{st}$ d of the experiment to study the effect on the acute as well as the chronic phase of the arthritis / it can be given from 14$^{th}$ d of the exp. to study its effect on the established arthitis.

# ANTI-INFLAMMATORY ACTIVITY – CHRONIC MODELS

- In vitro models
  - No single in vitro test is satisfactory
- Gastric Irritation test
  - This test is the most important side effect of antiarthritic drugs & any potential drug must be subjected to this test.
  - Test by Thuiller et al 1968 is considered most convenient & accurate test.

# ANTIALERGIC ACTIVITY

- Allergic condition such as bronchial asthma, atophica eczema, allergic rhinitis, etc affect about 20% pop & are increasing in prevalence & severity.
- Mouse & rat passive cutaneous anaphylaxis (PCA) test are convenient & reliable.

# TOXICITY EVALUATION

- Determined by the oral & intraperitoneal route in 2 / 3 adult albino of either sex & usually 15 – 20 g in weight.
- Test material is suspended in 0.1% agar / in 10% gum acacia in distilled water.
- A 20 g mouse receives 0.2 ml.
- Initial dose is at level of 400 – 500 mg/kg by a factor of 2.
- An interval of 1.5 is used 4 closer approximation.
- Doses higher than 1000 mg/kg are not generally used.
- The animal are observed for 5 to 6 h after dosage 4 toxic symptoms.
- If death occurs during this time, the cause of death is recorded.
- The approximate $LD_{50}$ is estimated & the maximum tolerated dose is also recorded.

# CLINICAL TRIAL

- Clinical trials new drug → mandatory b4 new drug is cleared for marketing
- It evaluates in 3 different phases.
- Phase I : the tolerability, kinetics, & metabolism of the test compound in different doses in healthy volunteers.
- Phase II: additional toxicity are carried out during this study such as: nephrotoxic, teratology, antigenecity, & mutagenecity.
- Phase III: include dose finding studies, food interaction studies, additional paraclincal such as: chronic toxicity & carcinogenicity are also conducted.

# ADDITIONAL INFORMATION

- Please read chapter 12 & 13 on book of Marine products for healthcare. Author: Venugophal, V.

# THANK YOU FOR ATTENTION